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Behavioral Performance, Brain Histology, and EEG Sequela After Immediate Combined Atropine/Diazepam Treatment of Soman-Intoxicated Rats

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PHILIPPENS, I. H. C. H. M., B. P. C. MELCHERS, D. M. G. DE GROOT AND O. L. WOLTHUIS. *Behavioral performance, brain histology, and EEG sequela after immediate combined atropine/diazepam treatment of soman-intoxicated rats.* PHARMACOL BIOCHEM BEHAV 42(4) 711-719, 1992. – It is known that rats poisoned with near-lethal doses of pinacolyl methylphosphonofluoridate (soman) develop brain lesions, particularly when convulsions are induced. When rats were intoxicated with a LD_{50} of soman and treated immediately thereafter with a combination of low doses of atropine and diazepam (LOW AS/DZ treatment), large decrements in performance of an earlier acquired shuttle-box task were found 6 days after intoxication. In contrast, no such decrements were found in soman-intoxicated animals treated similarly with a combination of high doses of these drugs (HIGH AS/DZ treatment). Surprisingly, surviving LOW AS/DZ animals acquired the same task again at a speed that was almost as fast as before intoxication. Similarly treated animals were examined lightmicroscopically 24 h after intoxication; in LOW-AS/DZ-treated animals, neuropathology was only observed in animals that had exhibited convulsions, whereas in HIGH AS/DZ animals neither convulsions nor brain damage were observed. Power spectra, obtained from electroencephalograms (EEGs) 6 days after intoxication, revealed significant differences between both treatment groups, particularly in the δ-, θ-, and β-frequencies. After the HIGH AS/DZ treatment, a significant increase in δ activity was found compared to control values, suggestive of neuropathology. It is concluded that, in contrast with the LOW AS/DZ combination, HIGH AS/DZ prevents active avoidance deficits, convulsions, and lightmicroscopically detectable neuropathology after soman intoxication. However, the results of EEG measurements suggest that some aberrations may still remain even after the HIGH AS/DZ treatment.

Behavior Brain lesions EEG Soman Atropine Diazepan

ADMINISTRATION of near-lethal doses of the organophosphorous cholinesterase inhibitor pinacolyl methylphosphonofluoridate (soman) causes neuropathology, predominantly in several cortical areas and in the hippocampus (13,15-20,22,23). Such lesions are not restricted to rats [see, e.g., (12)]. They are associated with long-term behavioral changes. In rats, 2 weeks after soman intoxication dose-dependent decrements were found in learning of an operant alternation task (20), whereas 3 weeks after intoxication deficits were reported in DRL acquisition (18), maze learning (23), and passive avoidance behavior (3). During this postexposure period, animals are hyperreactive to stimuli (17,23) and exhibit increased motor activity upon open-field testing (23), whereas after an LD_{50} of soman or higher other investigators (3) report decreased motor activity in the open field that becomes normal after 9 days. The effect on motor activity may be biphasic; the increase in motor activity may be preceded by an initial decrease (11). Such a decrease was earlier found to be dose dependent at low dose levels (29).

Diazepam (DZ), administered 10-15 min before soman, almost fully protects against the development of neuropathology without protecting against lethality (5,16). Atropine, when given alone in very high doses (40 mg/kg or higher) immediately after soman, also almost completely prevents the development of lesions [for references, see (8)]. On the other hand, when a range of combinations of atropine and diazepam were given 5 min after soman intoxication brain pathology was significantly reduced by all doses of diazepam and/

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or the highest dose of atropine, but no single drug or their combination was effective in protecting all animals in a group from some brain pathology (17).

In the present experiments, the effects of a high- or low-dose combination of atropine sulphate (AS) and DZ were tested and were called HIGH AS/DZ and LOW AS/DZ treatment, respectively. The rationale for this choice was that in our laboratory combinations of diazepam and atropine—at increasing dose levels—appeared to protect against lightmicroscopically detectable neuropathology in an increasing number of animals when administered immediately after soman intoxication (De Groot, personal communication).

The present experiments address the question in which way two selected treatment combinations, that is, HIGH AS/DZ or LOW AS/DZ, affect performance of soman-intoxicated rats of an earlier acquired shuttle-box task, quantified electroencephalogram (EEG), and histology of the brain.

METHOD

Animals

Male Small Wistar (WAG/MBL) SPF-reared rats with a starting body weight of 150–170 g and approximately 2 months old were used.

Experiment 1

Two groups of 16 rats each were subjected to active avoidance training in a computer-linked two-way shuttle-box as described before (26–29). Briefly, in 20 trials per day with an intertrial interval of 1 min (±20% random) animals were trained to avoid foot-shock (250 μA, constant-current principle) by moving into the other compartment within 10 s after a light stimulus was presented until they reached 80% or more correct avoidance responses (CARs) in two successive blocks of 10 trials. Subsequently, all animals were injected subcutaneously with soman (100 μg/kg). Immediately thereafter, half the number of animals in each group of 16 rats received the HIGH AS/DZ treatment (40 mg/kg AS + 2.5 mg/kg DZ) and the other half the LOW AS/DZ treatment (2 mg/kg AS + 0.625 mg/kg DZ). Treatments were administered by intraperitoneal injection.

From this moment on, animals were observed closely during the first 6 h and at different time intervals during the next 6 days to follow symptomatology. After this 6-day recovery period, animals were tested again during six daily sessions for their performance in the shuttle-box using the same procedure as before.

Experiment 2

Twenty-six animals were anesthetized with pentobarbital and a small hole was drilled in the skull, 6 mm caudal and 3.6 mm lateral of bregma, that is, over the caudal part of cortical area 17. The holes ended at the dura mater and a silver electrode of 0.4-mm diameter was fixed into the hole with dental cement. A silver reference electrode (connected to earth) was placed on the outside of the skull between the eyes and fixed in place (tip free) with dental cement.

Two days later, training of these animals started, following the same procedure described above. After reaching criterion at day 6, two groups were randomly formed.

For histology. As before, a group of 10 animals was injected with soman (100 μg/kg, SC); immediately thereafter, 5 animals received the HIGH AS/DZ treatment and the other 5 the LOW AS/DZ treatment. Animals were observed closely during the first 6 h after injections for the occurrence of tonic and/or clonic convulsions, tremors, chewing, or salivation. Twenty-four hours after injection, all animals in this group were anesthetized with ether and transcardially perfused with 4% formalin solution in water.

Based upon existing knowledge of the distribution and severity of soman-induced neuropathology, the tissue between olfactory bulbs and spinal cord of each animal was cut into different blocks. Embedding of the different blocks in paraplast was carried out in such a way that after microtomy the sections (3 μm thick) would show: a) predeliction areas, such as hippocampus and piriform cortex; b) areas with mild to moderate susceptibility to soman, such as caudate putamen and occipital cortex; and c) areas that are generally not affected, such as cerebellum and medulla oblongata. After staining with hematoxilin, azofloxin, and saffran (HAS), sections were examined lightmicroscopically in a "blind" fashion, that is, without knowing the treatment history of animals.

For neurophysiology and behavior. When animals had reached criterion and before injection with soman, control EEGs were obtained from each animal of a group of 16. The same technique was followed as used before (27,28): During EEG recording, animals were walking in a hollow Plexiglas wheel that rotated at a circumference speed of 10 cm/s and 5 epochs of 10 s were randomly chosen for fast Fourier transformation (FFT) to obtain a power spectrum.

Subsequently, animals were injected with soman (100 μg/kg, SC), immediately followed by the HIGH AS/DZ treatment in eight animals and the LOW AS/DZ treatment in the other eight.

As before, during the interval of 6 days that followed animals were closely observed during the first 6 h and at intervals later.

After this 6-day period of recovery, procedurally identical EEG recordings were again obtained from these animals just before the second training period of 6 days started.

Chemicals

Soman (>99% pure) was synthesized by Dr. H. P. Benschop from the Prins Maurits Laboratory TNO. Atropine sulphate and diazepam were obtained commercially. All solutions were freshly prepared before use.

Statistics

For statistical comparisons, the multiple *t*-test of Welch (21) was applied. When the term "significant" is used, this indicates a $p < 0.05$, tested two tailed.

RESULTS

Experiment 1

Immediately after intoxication and notwithstanding treatments, animals were severely motorically impaired; hence, the 6-day recovery period before performance was tested again in the second test period. Early during this time interval between the two test periods, four animals in the LOW AS/DZ group died; the remaining animals in that group recovered gradually and by the sixth day of the recovery period animals looked healthy again. Their body weights, measured immediately be-

fore injections and just before the second training period, were substantially reduced. In the HIGH AS/DZ group, the body weights decreased from 183 ± 3.0 g to 178 ± 10.7 g and in the LOW AS/DZ group from 179 ± 3.4 g to 157 ± 9.5 g.

Notwithstanding their healthy appearance, several animals had a sudden relapse during the second testing period and died rather suddenly: 6 of the 12 remaining survivors in the LOW AS/DZ group and 1 of 16 in the HIGH AS/DZ group.

In total, 1 animal in the HIGH AS/DZ group and 10 animals in the LOW AS/DZ group died. It is clear that HIGH AS/DZ treatment offers a significant protection. It should be emphasized that behavioral tests during the second test period were only carried out with animals that exhibited no overt symptoms whatsoever. Testing was omitted in the above-mentioned cases in which the condition of animals suddenly deteriorated and almost all died within a day.

In Fig. 1, it can be seen that after the 6-day recovery period animals in the HIGH AS/DZ group performed at the same level as before. In contrast, a significant performance decrement was found in the LOW AS/DZ group; in fact, animals started at approximately the same level as in the first session. Animals in the LOW AS/DZ group acquired the task again at roughly the same speed as before and in 6 days their performance reached the same level as that of the HIGH AS/DZ group. If only the performance of those animals that ultimately survive is taken into account, a similar picture emerges, albeit that the mean (±SEM) performance of the LOW AS/DZ group in session 7 then starts at 54 ± 13.6% (instead of 28 ± 10.3% as in Fig. 1) and the performance of the HIGH AS/DZ group starts at 79 ± 2.8% (instead of 74 ± 5.3%).

Since the presence of convulsions substantially aggravates brain damage, the data were regrouped on the basis of the presence or absence of convulsions, that is, regardless of the types of treatments. One animal in the HIGH AS/DZ group did and three animals in the LOW AS/DZ group did not exhibit convulsions. It can be seen in Fig. 2 that almost the same results are obtained as shown in Fig. 1. The improvement in performance of the LOW AS/DZ group in the second test period cannot only be ascribed to the fact that apparently only the least affected performers survived; the average score in session 7 was 37% CARs when only the performance of the three survivors in the group with convulsions was taken into account.

The number of escapes in both treatment groups was low and in the same order of magnitude (usually three to four per 20 trials). The number of intertrial responses (ITRs, changes of compartment during the intertrial period) in the HIGH

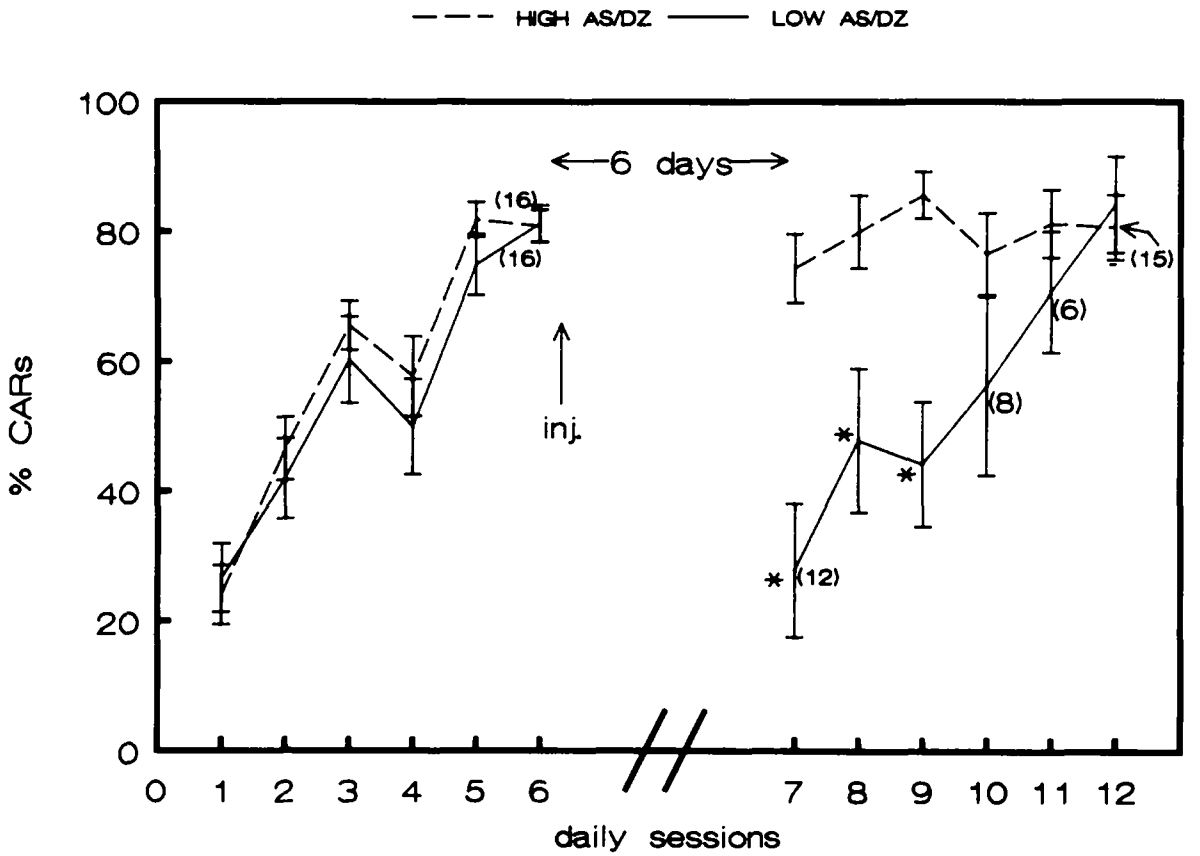

FIG. 1. Performance in a two-way shuttle-box of rats intoxicated with soman and treated immediately thereafter with a combination of atropine sulphate (AS) and diazepam (DZ) at high or low doses. After six daily training sessions, animals were injected on day 7 with soman (100 μg/kg, SC), immediately followed by IP treatment with either a combination of high doses of atropine sulphate (40 mg/kg) + diazepam (2.5 mg/kg) (HIGH AS/DZ) or low doses of atropine (2 mg/kg) + diazepam (0.625 mg/kg) (LOW AS/DZ). After a recovery period of 6 days, again six daily training sessions followed. Only animals that appeared healthy by observation were tested; their numbers are shown in the graph. *Significant ($p < 0.05$ two tailed) difference between treatment groups. Means ± SEM.

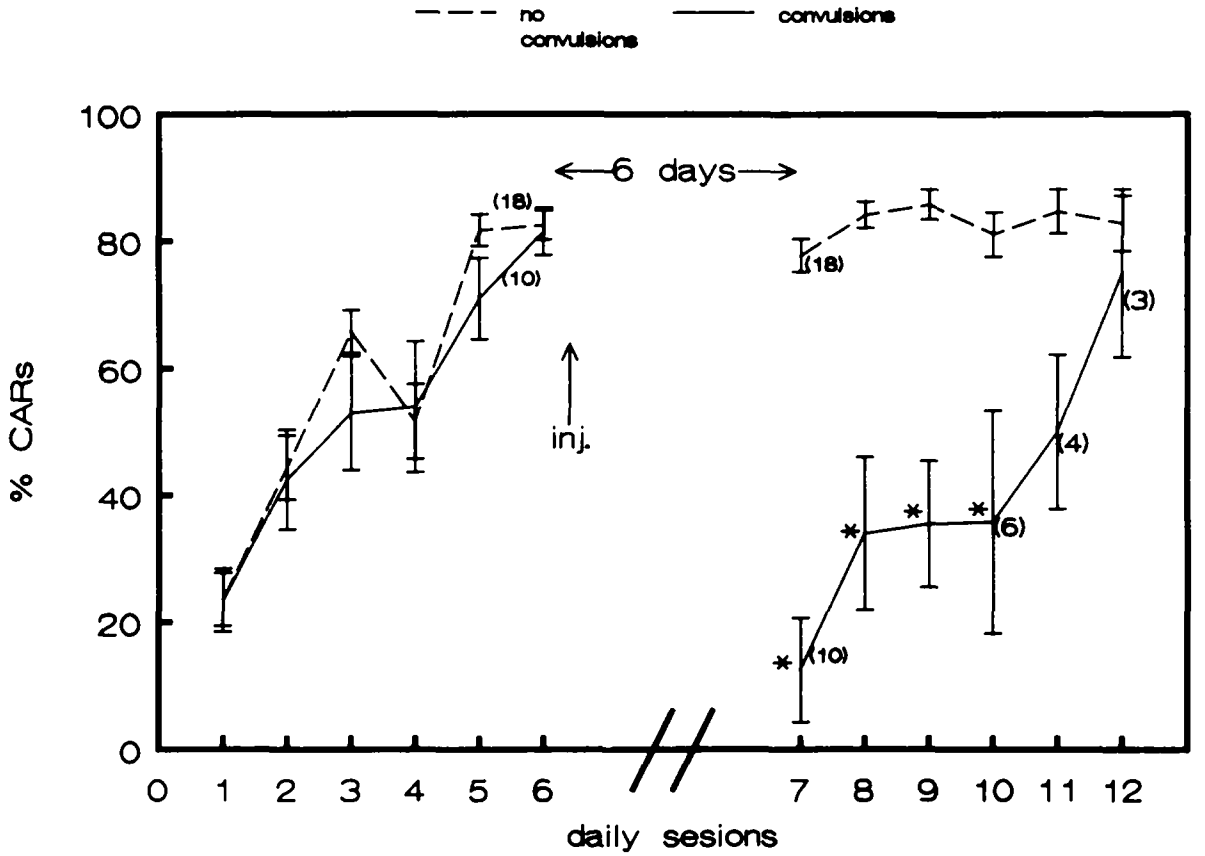

FIG. 2. Performance of the same animals as shown in Fig. 1, but here animals are regrouped on the basis of the presence or absence of convulsions, that is, regardless of treatment with high or low atropine/diazepam combinations.

AS/DZ group varied between 13-16 per session of 20 trials. In contrast, the number of ITRs of the LOW AS/DZ group increased rapidly from a mean (± SEM) of 22 ± 8.3 in session 7 to 62 ± 14.0 in session 12. This increase, as well as the difference between the two treatment groups, was significant. Whereas a low number of ITRs was not always associated with a low performance level, all animals with a high number of ITRs performed well.

Experiment 2

Histological examination of the CNS sections of rats in this experiment (see the Method section) showed that when convulsions were absent only minor—if any—histological changes could be observed. The morphology of nonconvulsing rats in the HIGH AS/DZ treatment group (five of five; see Figs. 3A, 3D, 4A, and 4D) was not different from that of nonconvulsing rats in the LOW AS/DZ treatment group (two of five; see Figs. 3B, 3E, 4B, and 4E). However, it was noted that a small number of "dark" (shrunken) and pyknotic cells were present not only in the predeliction areas such as the hippocampal CA3 area (see Figs. 3D and 3E) and the piriform cortex (see Figs. 4A and 4B) but also in other areas of the CNS, such as the occipital cortex (see Figs. 4D and 4E), that are usually less affected after soman intoxication. In rats of the LOW AS/DZ treatment group, three of five animals exhibited convulsions; in these rats, moderate to severe neuronal damage was observed, particularly in the hippocampal CA3 area (Fig. 3F) and the piriform cortex (Fig. 4C). In the CA3 area, severe edema and vacuolization was present in stratum lucidum, stratum radiatum, and stratum lacunosum. Nearly all pyramidal cells had a pyknotic appearance, indicating pronounced cell degeneration. Stratum oriens was less affected. The other hippocampal areas (CA1, CA2, and CA4) were also affected (see Fig. 3C) but showed far less damage: Mild neuropil edema was observed in stratum radiatum and stratum lacunosum moleculare and a number of pyramidal neurons had a dark appearance. However, the majority of the neurons looked "normal." The morphological changes in the distinct cortical areas differed; whereas all over the piriform cortex severe edema, vacuolization, and pyknosis (see Fig. 4C) was observed, the occipital cortex—for example—only showed mild to moderate edema, vacuolization, and pyknosis in layers 4-6, most pronounced in layer 6 (the polymorphic layer). Mild to moderate neuropathology was also present in the thalamic nuclei and the caudate putamen. No damage was observed in the cerebellum and the medulla oblongata.

Of the animals that remained to test active avoidance as well as for EEG recordings, all animals except one in the LOW AS/DZ treatment group, that is, seven of eight animals, had convulsions. In the HIGH AS/DZ treatment group none of the animals showed any convulsive activity. Their bodyweights, again measured just before the injections and 6 days later, increased from 186 ± 2.6 g to 188 ± 2.6 g in the HIGH

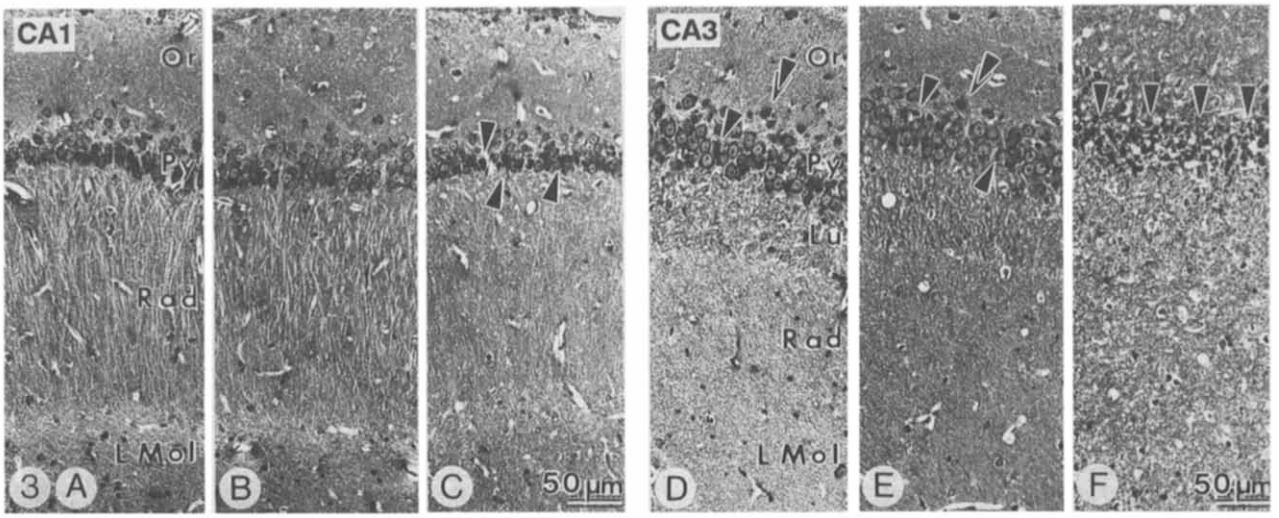

FIG. 3. Paraplast sections (3-μm thick) stained with hematoxilin, azofloxin, and saffran of the hippocampus of soman-intoxicated rats treated with two combinations of atropine sulphate (AS) and diazepam (DZ); see the legend of Fig. 1 for doses. A, CA1 area, nonconvulsing rat, HIGH AS/DZ treatment; B, CA1 area, nonconvulsing rat, LOW AS/DZ treatment; C, CA1 area, convulsing rat, LOW AS/DZ treatment; D, CA3 area, nonconvulsing rat, HIGH AS/DZ treatment; E, CA3 area, nonconvulsing rat, LOW AS/DZ treatment; F, CA3 area, convulsing rat, LOW AS/DZ treatment. Single arrowheads indicate pyknotic cells; double arrowheads indicate "dark" (shrunken) cells. Note that CA3 is more affected than CA1 by comparing F and C. In F, all neurons in striatum pyramidal (Py) are pyknotic and severe edema and vacuolization can be observed in stratum oriens (Or), stratum lucidum (Lu), stratum radiatum (Rad), and stratum lacunosum moleculare (L Mol). In C, a small number of neurons is pyknotic and only mild edema and vacuolization is visible in the other layers.

AS/DZ group and from 194 ± 2.3 g to 199 ± 2.8 g in the LOW AS/DZ group.

As can be seen in Fig. 5, essentially the same picture on shuttle-box performance emerges as in Fig. 1, albeit that in Experiment 2 the performance of animals receiving the HIGH AS/DZ treatment show a greater variability and only two of eight animals in the LOW AS/DZ group died during the second 6-day course of shuttle-box training. Both animals that died had exhibited long-lasting convulsions, and postmortem lightmicroscopic examination revealed that these two animals had severe lesions in the hippocampus and several cortical areas. Six days after injections, the averaged EEG power spectra (Fig. 6) of animals prior to behavioral testing showed that animals of the HIGH AS/DZ treatment group had developed a small δ-frequency peak around 2 Hz and a high degree of synchronization in the θ-frequency range around 8 Hz, both significantly different from the preinjection control values. In contrast, animals in the LOW AS/DZ treatment group had developed a large low-frequency peak (significantly larger than the peak in the HIGH AS/DZ group), whereas the peak around 8 Hz was significantly decreased, both when compared with the averaged power spectra of their own preinjection control EEGs or with the large synchronization peak of the HIGH AS/DZ group. In addition, EEGs of animals in the LOW AS/DZ group exhibited significantly lower β-frequency activity.

DISCUSSION

The difference in lethality between the two LOW AS/DZ groups of Experiment 1 (10 of 16 animals) and Experiment 2 (2 of 8 animals) was not significant and might be ascribed to seasonal variability in the sensitivity to soman. In both experiments, most animals died several days after intoxication. This late lethality is often seen after soman; it is an unexplained, poorly documented phenomenon that has long been known to insiders as "the late death syndrome" and might be the ultimate result of brain lesions and/or cardiac pathology (17).

In view of the fairly large histological data-base on the type, location, and gravity of soman-induced neuropathology (for references, see the introductory section), the brains of only two groups of five animals were histologically examined in the present investigation (see Figs. 3 and 4).

Only in the three animals of the LOW AS/DZ treatment that exhibited convulsions was clear neuropathology found. In all other animals, lightmicroscopically detectable neuropathology was virtually absent, perhaps with the exception of a few pyknotic cells (see also below), which are also found in areas that are usually only slightly affected by soman. In addition, the two animals in Experiment 2 that died spontaneously during the second period of avoidance training (at 9 and 10 days after intoxication, respectively; see Fig. 5) had experienced convulsions and exhibited gross neuropathology.

Earlier, McLeod et al. (19) also found consistent neuropathology in animals that had experienced convulsions and no lightmicroscopial lesions in animals without convulsions. Similar findings were reported in a detailed study by McDonough et al. (17), albeit these authors also found minimal to mild lesions in two of seven animals that had not experienced convulsions. For the purpose of the present article, therefore, it seems reasonable to accept that convulsing rats develop lesions. This does not exclude that animals that do not convulse may have small lesions that might be functionally important and may or may not be reversible. Such lesions may only be detectable by electron-microscopical analysis. Whether the few pyknotic cells in the hippocampus and cerebral cortex (see the Results section) have any significance and whether they are the result of the soman intoxication or due to the preparation procedure remains to be answered. This question is not irrele-

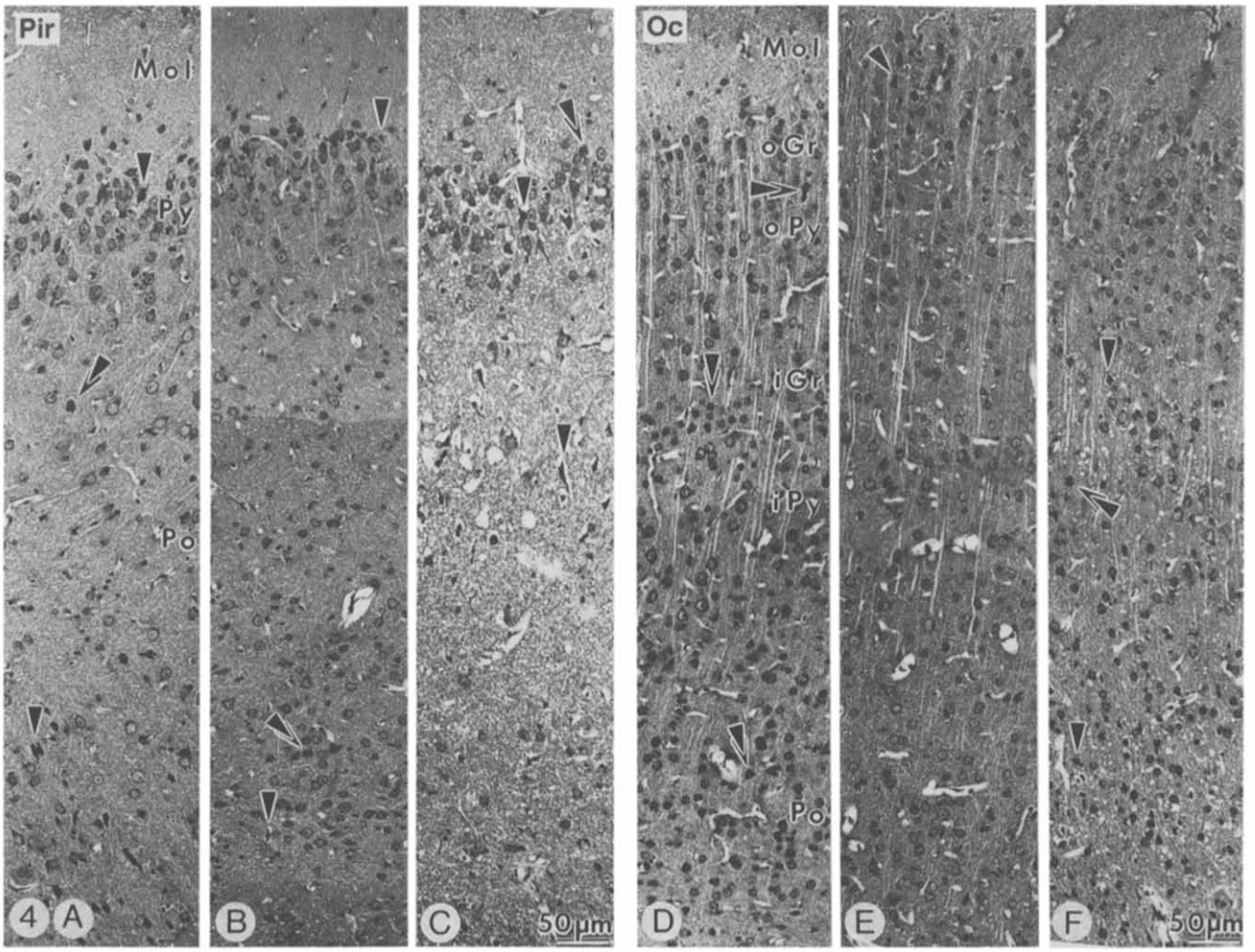

FIG. 4. Paraplast sections (3-μm thick) stained with hematoxilin, azofloxin, and saffran from the cerebral cortex of soman-intoxicated rats and treated with two combinations of atropine sulphate (AS) and diazepam (DZ); see the legend of Fig. 1 for doses. A, piriform cortex, nonconvulsing rat, HIGH AS/DZ treatment; B, piriform cortex, nonconvulsing rat, LOW AS/DZ treatment; C, piriform cortex, convulsing rat, LOW AS/DZ treatment; D, occipital cortex, nonconvulsing rat, HIGH AS/DZ treatment; E, occipital cortex, nonconvulsing rat, LOW AS/DZ treatment; F, occipital cortex, convulsing rat, LOW AS/DZ treatment. Single arrowheads indicate pyknotic cells; double arrowheads indicate "dark" shrunken cells. Note that the piriform cortex (Pir) is much more affected than the occipital cortex (Oc) by comparing C and F. In C, severe cell pyknosis, edema, and vacuolization is seen, particularly in the polymorphic layer (Po). The molecular (Mol) and pyramidal (Py) layers, although clearly damaged, are less affected. In F, edema and vacuolization is observed, particularly in the inner granular (iGr), inner pyramidal (iPy), and polymorphic (Po) layers. The molecular (Mol) and outer granular (oGr) and pyramidal (oPy) layers are not or hardly affected.

vant since the results of the present EEG measurements in the HIGH AS/DZ group (see below) indicate that, notwithstanding the absence of convulsions, significant aberrations may still be present in the EEG 6 days after intoxication, suggestive of persisting neuropathology.

Apart from neuronal death, the EEG changes might also result from a loss of muscarinic receptors (7) that also occurs in vitro (2) from a slowly reversible intracellular process such as has been found at peripheral (nicotinic) cholinergic sites (1) or from persisting neuropile edema and disappearance of dendritic spines (6).

To assess the behavioral effects (Figs. 1, 2, and 5) of soman intoxication followed by the two dose-wise different atropine/diazepam treatments, a two-way shuttle-box technique was used since previous experiments have demonstrated that performance of this conditioned avoidance task was very sensitive to cholinergic manipulation with cholinesterase inhibitors (28,29). An interesting finding in these experiments was not only that the HIGH AS/DZ treatment prevented the behavioral decrements that were found in the LOW-AS/DZ-treated group but also that the latter animals could again acquire the task at a speed that did not substantially differ from the original speed of learning. Since animals in the present experiments were tested for their performance of the same task they had acquired before intoxication, the results make it highly likely that in the LOW AS/DZ groups specifically memory or retrieval mechanisms were affected and not the ability to learn this task (again). On the basis of the literature (3,11,13, 18,20,23), a distinction between acquiring or memorizing a task cannot be made since animals had to learn a task after a

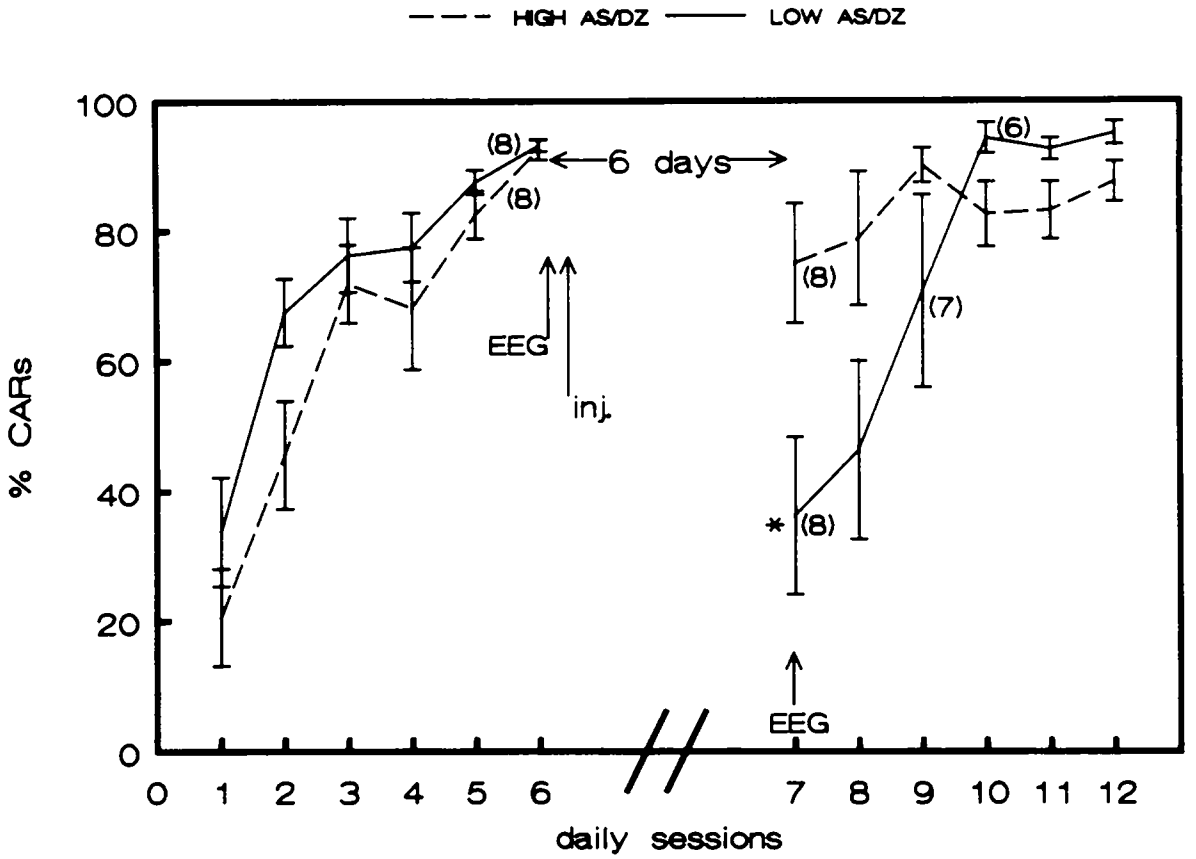

FIG. 5. Performance in a two-way shuttle-box of rats in Experiment 2. The two groups of animals were intoxicated and treated in the same way as those in Fig. 1 with the exception that they had been fitted with EEG electrodes 2 days prior to the start of the experiment. EEG measurements were carried out just before intoxication and 6 days later, just before the second period of training started. Their EEG spectra are shown in Fig. 6. Number of animals between brackets. *Significant ($p < 0.05$, two-tailed) difference between treatment groups. Means ± SEM.

recovery period following soman intoxication; the inability to perform such a new task may be caused by either learning or memory deficits as a result of soman intoxication.

An explanation for the differences between the present results and those of McDonough et al. (17), who found lesions as well as behavioral deficits, may also be that in their case the treatment with different combinations of atropine and diazepam started 5 min after intoxication, whereas in the present experiments treatment was administered immediately after intoxication in an attempt to prevent convulsions. Taking the results of McDonough et al. (17) and the present results together, it seems likely that in the 5-min time interval a process has started that leads to the development of neuropathology. If true, the present results support the statement by McDonough et al. (17) that pharmacological management of epileptiform motor abnormalities during acute intoxication is critical for the development of brain lesions and, in our view, also for the development of behavioral abnormalities. The earlier anticonvulsive treatment is administered, the better. In addition, the dose of atropine in the present HIGH AS/DZ group was higher and the dose of diazepam somewhat lower than the highest doses used by McDonough et al. (17), which was the result of experiments in our laboratory indicating that high doses (40 mg/kg or higher) of atropine alone could almost completely prevent the development of lightmicroscopically detectable lesions [for references, see (8)].

The large increase in the number of intertrial responses in the LOW AS/DZ treatment group is difficult to interpret without further experimental data. These intertrial responses are usually taken as a measure of motor activity. If true, this effect might be due to brain cortex lesions, causing a release of behavioral inhibition, which in turn may be responsible for the increased reactivity to stimuli and the increased motor activity as reported by several authors (11,17,23). However, whatever its origin this increase in intertrial responses excludes the possibility that performance drops because animals are motorically impaired or too ill to move.

The EEG differences (see Fig. 6) between the two treatment groups suggest that particularly the increase of the low-frequency peak in the δ range is indicative for the presence of lesions. In earlier experiments in our laboratory under similar conditions (28) using single or repeated low doses (60 μg/kg SC) of soman, such low-frequency peaks were observed 30 min after this organophosphate, but had disappeared 24 h later. The finding that in animals in the LOW AS/DZ treatment group a significant low-frequency peak persists for at least 6 days after a single dose of 100 μg/kg soman suggests the presence of permanent damage. Moreover, the significant enhancement of δ activity in the EEGs of the HIGH AS/DZ treatment group, albeit significantly less than in the EEGs of the LOW AS/DZ treatment group, suggests that also some neuropathology is present in the HIGH AS/DZ group. The

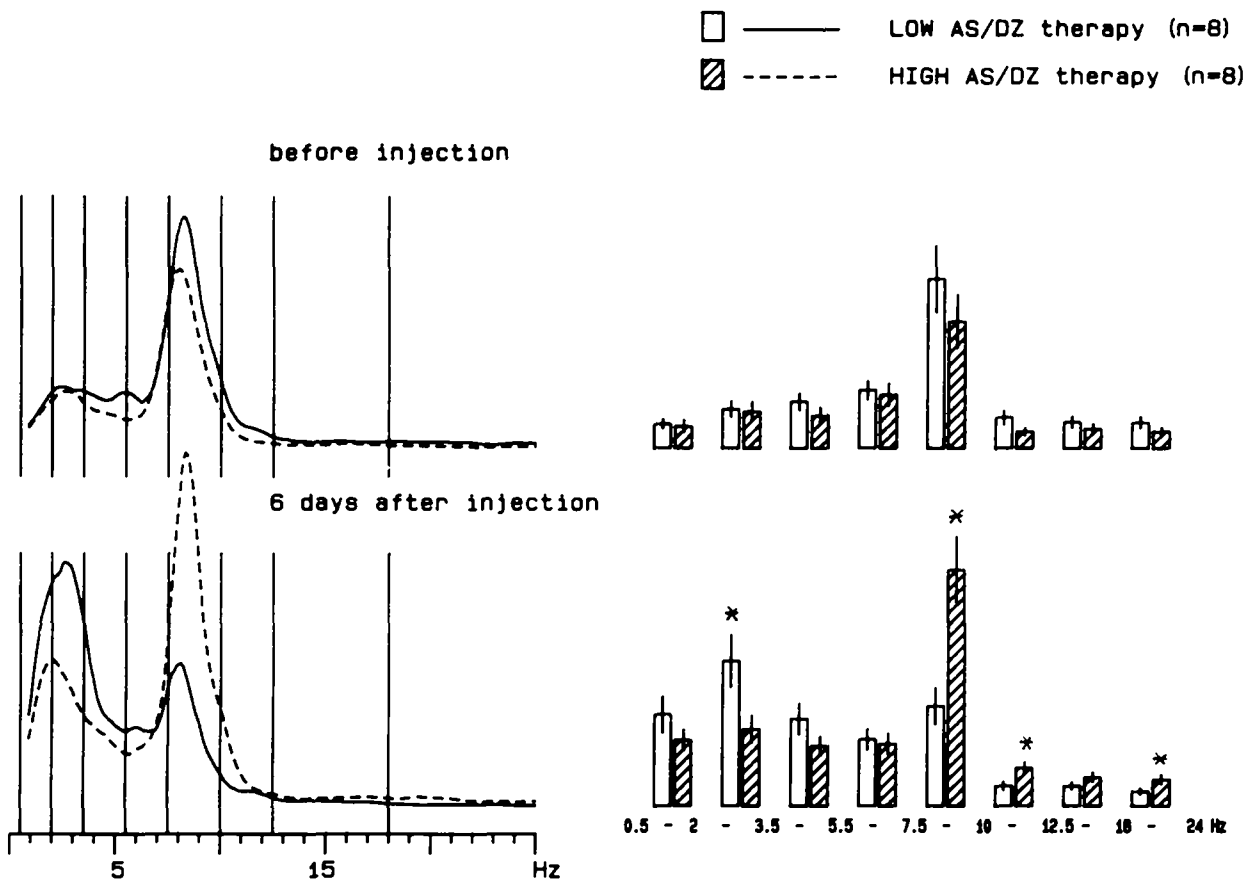

FIG. 6. Average power spectra of animals shown in Fig. 5. The control spectra in the upper panel were obtained before injections, whereas the lower panel represents the spectra obtained from those animals that had been treated, after intoxication 6 days earlier, with either a combination of high doses of atropine and diazepam (HIGH AS/DZ) or low doses of atropine and diazepam (LOW AS/DZ). The curves represent the continuous spectra and are subdivided by vertical lines to delineate frequency classes. The bars (± SEM) show the result for each frequency class. Excursions in the vertical direction represent the power (V^2) in arbitrary units.

differences between the two treatment groups in the dominant frequency around 8 Hz (θ) of these walking rats is hard to explain; in the HIGH AS/DZ group, a significantly increased peak in the θ range was found, whereas following the LOW AS/DZ treatment the peak around this frequency was significantly reduced when compared with preinjection control values. Such a high degree of synchronization was also observed in earlier experiments (28) 24 h after a single or multiple sign-free doses of soman or DFP. Therefore, an increased θ peak in this case might reflect increased cortical cholinergic stimulation due to persisting cholinesterase inhibition, whereas a decreased θ peak might represent a decrease in cholinergic stimulation due to lesions of acetylcholine-producing cells. Undoubtedly, such an explanation is an oversimplification.

The EEGs of the LOW AS/DZ group show a significant reduction of β activity compared with those of the HIGH AS/DZ group, which might also point to persisting lesions. Compared with preinjections values, there is a tendency toward a decrease of β activity in the LOW AS/DZ group and a tendency toward increased β activity in the HIGH AS/DZ group. However, the latter increase is only significant at the frequency band between 10–12.5 Hz (β_1 activity).

Increased δ activity in awake animals was observed earlier after cobalt cortical implants and after large doses of alcohol (14). Its presence is thought to be associated with behavioral impairment (10,25). In a study of the long-term effects on the (visually inspected) EEG of sarin-exposed humans (9), increased δ and θ slowing, as well as increased β activity, was noted. In a later study of a similar nature by the same authors that included also acutely and chronically sarin-exposed rhesus monkeys, the major finding (upon EEG spectral analysis) was an increased β activity in the EEGs of the sarin-exposed population (4). Quisqualic or ibotenic acid lesions of the nucleus basalis in rats (24), with its major cholinergic projections to the cortex, give rise to increased δ and decreased β activity similar to the present LOW AS/DZ group. However, unlike the EEGs of the present LOW AS/DZ group, an increase in θ activity was also found.

In short, a clear and precise explanation of the present EEG findings cannot be given on the basis of these and other literature data studied, the main reason being that in the present experiments the electrical activity of the cortex, 6 days after intoxication, may be the mixed result of a) cholinergic stimulation, b) the involvement of other transmitters, and c) neuropathology. However, it seems likely that the increase in δ activity found is indicative for neuropathology.

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